Systemic Estrone Production and Injury-Induced Sex Hormone Steroidogenesis after Severe Traumatic Brain Injury: A Prognostic Indicator of Traumatic Brain Injury-Related Mortality

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Abstract

Extensive pre-clinical studies suggest that sex steroids are neuroprotective in experimental traumatic brain injury (TBI). However, clinical trials involving sex hormone administration have not shown beneficial results, and our observational cohort studies show systemic estradiol (E2) production to be associated with adverse outcomes. Systemic E2 is produced via aromatization of testosterone (T) or reduction of estrone (E1). E1, also produced via aromatization of androstenedione (Andro) and is a marker of T-independent E2 production. We hypothesized that E1 would be (1) associated with TBI-related mortality, (2) the primary intermediate for E2 production, and (3) associated with adipose tissue-specific aromatase transcription. We assessed 100 subjects with severe TBI and 8 healthy controls. Serum levels were measured on days 0–3 post-TBI for key steroidogenic precursors (progesterone), aromatase pathway intermediates (E1, E2, T, Andro), and the adipose tissue-specific aromatase transcription factors cortisol, tumor necrosis factor-alpha (TNF-α), and interleukin-6 (IL-6). E1 was elevated after TBI versus controls. High E1 was associated with higher progesterone, cortisol, and IL-6 ($p < 0.05$). Multivariable logistic regression demonstrated that those in the highest E1 tertile had increased odds for mortality (adjusted OR = 5.656, 95% CI = 1.102–29.045, $p = 0.038$). Structural equation models show that early serum E2 production is largely T independent, occurring predominantly through E1 metabolism. Acute serum E1 functions as a mortality marker for TBI through aromatase-dependent E1 production and T-independent E2 production. Further work should evaluate risk factors for high E2 production and how systemic E2 and its key intermediate E1 contribute to the extracerebral consequences of severe TBI.

Keywords: aromatase; estradiol; estrone; inflammation; TBI

Introduction

Traumatic brain injury (TBI) contributes to $\sim$235,000 hospitalizations and 50,000 deaths annually in the United States.[1] The complex and multifaceted nature of secondary damage following the primary insult that occurs with TBI is a major issue that has made identifying efficacious treatments difficult.[2,3] In addition, the systemic pathophysiological responses to TBI, as well as concomitant extracerebral injury and non-neurological organ dysfunction (NNOD), have been relatively underappreciated for their role in affecting TBI outcome.[4–8] Further, the autonomic nervous system response to injury may be central to at least a portion of the systemic pathology associated with NNOD after TBI, including the initiation of an innate inflammatory response,[8–10] which we posit has some influence on other systemic response pathways such as injury-induced steroidogenesis.[11]

Multiple animal studies suggest that sex hormones such as progesterone and estradiol (E2) mitigate damage in models of TBI[12–15] and stroke.[15–18] However, systemic E2 production has been associated with poor outcomes and higher mortality rates in critical illness,[19,20] major trauma,[21,22] sepsis,[23–26] nontraumatic subarachnoid hemorrhage,[27] and severe TBI.[11] The steroidogenesis of estrogens

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begins with progesterone conversion to the sex steroid androstenedione (Andro), which then may be metabolized further in multiple tissue types, including adipose tissue, to E2 via aromatization and reduction pathways using testosterone (T) and estrone (E1) as steroidogenic precursors (Fig. 1). Andro is converted to E2 through two major steroidogenic pathways. The first is a T-dependent pathway in which Andro is reduced predominantly via 17β-hydroxysteroid dehydrogenase-3 (17β-HSD-3) to T and then aromatized via the aromatase enzyme to E2.[28,29] The second is a T-independent pathway requiring aromatization to E1 followed by reduction to E2 using enzymatic reductase isoforms 1 and 7 (17β-HSD-1,7).[28,29]

T has a much lower affinity for aromatase than Andro;[30] therefore, recent reports suggest that estrogenic steroidogenesis, particularly in extragonadal tissues, favors biosynthetic pathways that do not consume T.[30] In addition to elevated serum E2 levels in both men and women with major illnesses, concurrent T consumption is observed specifically in men and postmenopausal women.[20,23,24,31,32] Increased systemic aromatization in response to post-injury stress may occur via multiple transcription factors that include tumor necrosis factor-alpha (TNF-α),[33,34] cortisol,[35] and interleukin-6 (IL-6),[36] and may support both T and E1 consumption for E2 production.[37] Therefore, we hypothesize that E1 may be a prognostic biomarker for mortality after severe TBI that also reflects/contributes to NNOD, along with other factors such as hypothalamic–pituitary axis (HPA) activation and injury-induced initiation of the systemic inflammatory response.

Regardless of whether T-dependent or T-independent aromatization occurs, serum E2 levels are associated with higher mortality risk in the setting of acute stressors such as critical illness and severe trauma.[19,22,38] Higher serum E2 and T levels acutely after TBI have both been associated with acute mortality and poor 6 month Glasgow Outcome Scale (GOS) scores in patients with severe TBI.[11] Further, T pretreatment in animal models of TBI can increase histological damage.[39]

Despite being a T-independent precursor to E2, little is known about E1 effects in brain injury pathology and outcome. Similar to other E2 administration studies involving experimental central nervous system (CNS) injury models,[40–44] rat TBI models and cellular neuronal injury models suggest that supraphysiological E1 administration can be neuroprotective.[45–47] Akin to clinical studies evaluating endogenous systemic E2 levels, clinical studies in septic shock and subarachnoid hemorrhage draw a sharp contrast to these experi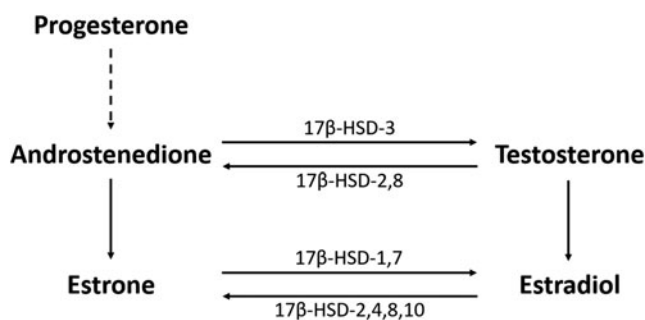mental findings of E1-associated neuroprotection by showing that increased endogenous systemic E1 levels are associated with worse outcomes.[27,48] E1 associations with septic shock are interesting when considered in the context of TBI, given that NNOD following TBI can lead to systemic inflammation[49] and immune dysregulation.[50] Further, inflammatory mediators are associated with increased aromatase activity,[36,51] which may act to increase E1 production.[30] Together, E1's association with outcomes in other types of brain injury, along with its role in sex hormone steroidogenesis, make it a potential systemic biomarker of injury when predicting outcomes following TBI and when understanding possible contributions to NNOD associated with TBI. Therefore, the goals of this study are to (1) identify if E1 is elevated in TBI, (2) determine if E1 can predict outcomes following TBI, and (3) assess E1's association with other biomarkers and sex hormones known to be dysregulated after TBI.

Methods

Study design and subjects

This study was approved by the University of Pittsburgh's Institutional Review Board. We enrolled 100 unique individuals admitted with severe TBI, defined as admission Glasgow Coma Scale (GCS) ≤8, at a level 1 trauma center. Figure 2 shows how our cohort was used for analysis: 96 patients had E1 data from days 0–3, and 92 of these individuals had E1, T, E2, and Andro data from samples collected on days 0–3 post-injury. Inclusion criteria were as follows: (1) age between 16 and 75 years, (2) positive findings on head CT, (3) insertion of extraventricular drainage catheter (EVD) for intracranial pressure (ICP) monitoring, and (4) signed consent for enrollment from appropriate proxy. Individuals with penetrating brain injury, documented history of prolonged cardiac or respiratory arrest, history of endocrine tumor, history of breast cancer requiring chemotherapy or tamoxifen, history of prostate cancer with orchiectomy or luteinizing hormone suppressing agents, or untreated thyroid disease were excluded from this study. Also, eight healthy controls were recruited as a reference group for serum hormone measurements. Healthy controls did not have previous endocrine pathology, were not pregnant, and were not using oral contraceptives, hormone replacement therapy, or hormone-modifying supplements. Those with a history of TBI, neurological disorder, or bleeding disorder were also excluded as healthy controls.

FIG. 1. Sex hormone steroidogenesis from progesterone. Dashed line represents conversion of progesterone to 17α-hydroxyprogesterone and subsequently to androstenedione via cytochrome P450c17. The main isoforms of 17β-hydroxysteroid dehydrogenase (17β-HSD) catalyzing oxidative and reductive pathways are shown.
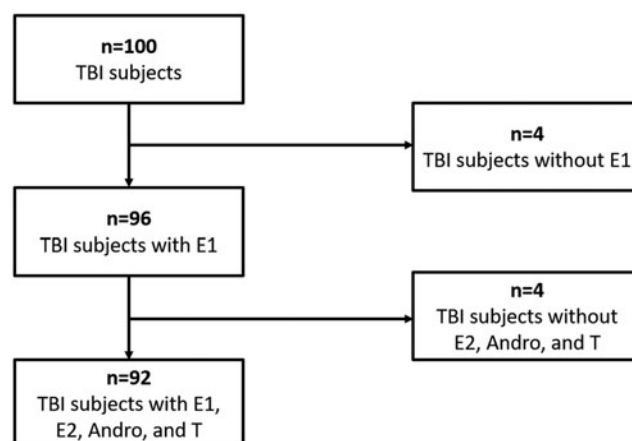

FIG. 2. Divisions of cohort used for analyses. Our 100 patient cohort had 96 individuals with data on E1 and 92 individuals with data on estrone (E1), estradiol (E2), androstenedione (Andro), and testosterone (T).

Critical care management of individuals with TBI was consistent with *The Guidelines for the Management of Severe Head Injury*.[52] Initial interventions included targeted temperature management, EVD placement, central venous catheterization, arterial catheterization, and monitoring via pulse oximetry. ICP and cerebral perfusion pressure (CPP) were managed to target (< 20 mm Hg and >60 mm Hg, respectively). Mean arterial pressure was maintained at least at >90 mm Hg. Two patients were enrolled in a trial evaluating moderate hypothermia in TBI, and five patients were involved in the Citicoline Brain Injury Treatment (COBRIT) study. The details of these trials are as described previously.[53]

Study variable description

The clinical and demographic characteristics of the individuals with E1 data ($n = 96$) were assessed in relation to survival status and day 0–3 E1 levels. Demographic variables (age, sex, race, body mass index [BMI]) were recorded from admission hospital records. We obtained best in 24 h GCS, given that it more reliably predicts cognitive outcome than does admission GCS.[54,55] Additional clinical variables included Injury Severity Score (ISS), non-head ISS, length of hospital stay (LOS), mechanism of injury (MOI), total hospital complications (e.g., pulmonary, cardiac), and injury type on head CT. The vast majority of subjects (or proxy respondents) could not provide accurate information regarding hormone supplementation/replacement at the time of injury.

The GCS is a measure of the degree of impaired consciousness in patients, with lower scores representing more severe deficits.[56] The ISS combines scores from the most severe injuries in distinct anatomical locations, resulting in a positive association with injury severity.[57] We additionally calculated a non-head ISS, excluding brain injuries, using the same method to represent extracerebral trauma severity. Injury types on head CT included subdural hemorrhage (SDH), subarachnoid hemorrhage (SAH), diffuse axonal injury (DAI), epidural hemorrhage (EDH), contusion, intraventricular hemorrhage (IVH), intracerebral hemorrhage (ICH), and other injury not classified elsewhere. CT injury types associated with mortality in bivariate analysis ($p < 0.2$) were incorporated into a neurological burden score (NBS). NBS was calculated by summing the total number of these injuries observed on CT in each patient. Presence of SDH, SAH, and contusion were summed to calculate the NBS. Presence of sepsis, splenic injury, and other abdominal injury was also obtained using records on International Statistical Classification of Disease Injury codes version 9 (ICD-9). Presence of sepsis, splenic injury, and other abdominal injury were summed to generate a score (SSA) to assess concomitant extracerebral inflammatory burden. We measured mortality at 1 month given that the majority of deaths after TBI occurred in the 1st month in our cohort and a previously reported mean acute hospital stay of 3–5 weeks following severe TBI.[58–60]

Serum sample collection and measurements

Blood samples were collected at ∼7:00 a.m. daily during days 0–3 post-injury. Samples were centrifuged, aliquoted, and stored at −80°C until used for assay. Samples from control subjects were drawn at 7:00 am, processed, and stored as described. Serum samples were measured for E2 ($n = 272$ samples), T ($n = 272$ samples), and progesterone ($n = 281$ samples), using radioimmunoassays via the COAT-A-COUNT® In-vitro Diagnostic Test Kit (#TKE2, #TKTT, and #TKPG, respectively; Siemens Healthcare Diagnostics Inc., Tarrytown, NY). Assay sensitivities were as follows: E2, 8 pg/mL; T, 4 ng/mL; and progesterone, 0.03 ng/mL. Serum samples ($n = 212$) were measured for Andro using competitive enzyme-linked immunosorbent assay (ELISA) kits (sensitivity 0.021 ng/mL; #EIA-3265; DRG International, Springfield, NJ). Also, serum samples ($n = 202$) were measured for E1 con-

centrations using competitive ELISA kits (sensitivity 2.21 pg/mL; #EIA-4174; DRG International, Springfield, NJ).

Given the known effects of cortisol and inflammatory cytokines as aromatase gene transcription factors within the steroidogenesis pathway,[35,36,51] we assessed cortisol, TNF-α, and IL-6 levels measured in this population. Serum samples were measured for cortisol ($n = 281$ samples) using RIA via the COAT-A-COUNT® In-vitro Diagnostic Test Kit (sensitivity 20 ng/mL; #TKCO; Siemens Healthcare Diagnostics Inc., Tarrytown, NY). Serum samples ($n = 201$) were measured for TNF-α, and IL-6 concentrations using a Luminex™ bead array assay (#HSCYTO-60SK; Millipore, Billerica, MA). Sensitivities for the cytokines were as follows: TNF-α, 0.05 pg/mL; and IL-6, 0.1 pg/mL.

Statistical analysis

Statistical analyses were completed using SPSS (v23.0; IBM, Armonk, NY) and SAS (v9.4; SAS Institute, Cary, NC). Summary statistics of means, standard error of means (SE), medians, and interquartile ranges (IQR) were calculated for continuous variables. Data were assessed for normality using the Shapiro–Wilk test, and nonparametric tests (Mann–Whitney's test, Kruskal–Wallis's test, and Spearman's rank correlation) were used as appropriate to compare variables when data did not meet normality assumptions. Categorical variables were summarized with frequencies and percentages. Associations between categorical variables were determined using the χ^2 test, or the Fisher's exact test as appropriate.

For all individuals with TBI and serum E1, averages were calculated. Restricted cubic spline (RCS) analysis was conducted to assess departures from linearity in the effect of E1 on mortality over the range of E1 levels in the study population. The RCS procedure uses piecewise functions of low-order polynomials over specified intervals, known as knots, to discern points of departure from linearity.[61] After inspecting these data, we applied tertile cut points for E1 to discriminate the dose-response relationship between E1 and mortality. Subjects were accordingly assigned to E1 low, medium, and high groups based on having E1 levels in the 0th–25th, 25th–75th, and 75th–100th percentiles.

Binary logistic multivariate regression was performed to adjust for the effects of demographic and injury variables in assessing the association between E1 tertile and 1 month mortality. Covariates were tested in the model if they demonstrated a trend with mortality ($p < 0.2$). To assess potential variation between hormone values by sex, interaction terms were generated between sex and E1 level.

Day 0–3 averages for individuals with Andro, T, E2, and E1 were calculated for 92 patients. Mean hormone levels in relation to mortality status were assessed as well as average Andro, T, and E2 levels for each E1 tertile. In order to assess relationships between serum steroids in the aromatization pathway via causal inferences, a structural equations model (SEM) was fitted using the covariance analysis of linear structural equations (CALIS) procedure in SAS. The data for the four serum hormones were first z-score standardized to have a mean of 0 and a standard deviation of 1 to adjust for inherent differences between hormone marker concentrations. An SEM was run among all individuals, and a post-hoc exploratory analysis was conducted among survivors and non-survivors respectively. Standardized β coefficients and standard errors were reported for each path, and significant paths ($p < 0.05$) were indicated.

Results

Characteristics of cohort

Demographic and clinical characteristics by survival status of the 96 enrolled subjects with data on days 0–3 are described

in Table 1. Mean age of the cohort was 37.3 ± 1.7 years, and mean BMI was 25.8 ± 0.5. The majority of the cohort were men (83.3%) and white (92.7%). The primary mechanism of injury was motor vehicle collision (50%) followed by motorcycle accidents (18.8%) and falls (16.7%). Mean LOS was 19.8 ± 1.3 days, and median total number of hospital complications was 1 (IQR 1–2).

In our cohort, 29 subjects (30.2%) did not survive past 1 month. Non-survivors were on average 12.3 years older (mean age 45.9, $p < 0.001$) and had a shorter LOS by 14.4 days (mean 9.8, $p < 0.001$). Presence of SDH, SAH, and contusion were summed to calculate the NBS. Of injury types on CT, SAH and contusions were more frequent among non-survivors (89.7%, $p = 0.021$ and 65.5%, $p = 0.011$, respectively). There also was a trend for SDH to occur more frequently among non-survivors as well ($p = 0.099$). Sex, race, BMI, best in 24 h GCS, ISS, and non-head ISS were not associated with 1 month mortality status. In addition, sepsis, splenic laceration, abdominal injury, and SSA score were not associated with mortality.

Daily E1 levels by mortality status, sex, and age

Average daily E1 levels (for days 0–3) were compared between subjects surviving at least 1 month and those who did not survive 1 month following injury (Fig. 3A). E1 was significantly elevated compared to control values for both survivors and non-survivors ($p < 0.02$ all comparisons). E1 was elevated on days 2 and 3 in non-survivors compared with survivors ($p < 0.05$ in both comparisons). Daily E1 levels were also examined by sex (Fig. 3B). E1 also was elevated in both men and women with TBI compared with controls ($p < 0.02$ both comparisons). Whereas values for women rose over time, values for men decreased after day 1 such that pairwise analysis showed that E1 levels were significantly higher by day 3 in women compared to in men ($p < 0.02$). Daily E1 levels were also examined by age, comparing subjects ≤35 to those >35 years of age (Fig.3C). Age 35 was chosen as the cutoff to reflect the median age of our cohort, and E1 levels were higher in TBI subjects than in controls regardless of age ($p < 0.05$ all comparisons). However, no

TABLE 1. POPULATION CHARACTERISTICS BY SURVIVORSHIP AT 1 MONTH

	Total (n=96)	Survivors (n=67)	Non-survivors (n=29)	p value
Age, mean (SE)	37.3 (1.7)	33.6 (1.9)	45.9 (3.2)	<0.001[a]
Sex, men (%)	80 (83.3)	58 (86.6)	22 (75.9)	0.196
Race, n (%)				0.794
Black	6 (6.3)	4 (6.0)	2 (6.9)	
White	89 (92.7)	62 (92.5)	27 (93.1)	
Other	1 (1.0)	1 (1.5)	0 (0.0)	
BMI, Mean (SE)	25.8 (0.5)	25.6 (0.6)	26.4 (1.1)	0.480
GCS (best in 24 h), median (IQR)	6 (5–7)	7 (5–8)	6 (4.5–7)	0.187
ISS, Mean (SE)	33.9 (1.0)	32.9 (1.2)	36.3 (1.8)	0.230
Non-head ISS, mean (SE)	11.7 (1.0)	11.2 (1.2)	12.9 (2.0)	0.600
Hospital length of stay, mean (SE)	19.8 (1.3)	24.2 (1.5)	9.8 (1.4)	<0.001[a] [b]
Mechanism of injury, n (%)				
Motor vehicle accident	48 (50.0)	36 (53.7)	12 (41.4)	
Motorcycle	18 (18.8)	14 (20.9)	4 (13.8)	
Falls	16 (16.7)	6 (9.0)	10 (34.5)	
Assault	5 (5.2)	4 (6.0)	1 (3.4)	
Other	6 (6.3)	5 (7.5)	1 (3.4)	
Unknown	3 (3.1)	2 (3.0)	1 (3.4)	
Injury type, n (%)				
SDH	61 (63.5)	39 (58.2)	22 (75.9)	0.099[c]
SAH	71 (74.0)	45 (67.2)	26 (89.7)	0.021[a]
DAI	33 (34.4)	27 (40.3)	6 (20.7)	0.063[c]
EDH	17 (17.7)	12 (17.9)	5 (17.2)	0.937
Contusion	44 (45.8)	25 (37.3)	19 (65.5)	0.011[a]
IVH	31 (32.3)	26 (38.8)	5 (17.2)	0.038[a]
ICH	33 (34.4)	24 (35.8)	9 (31.0)	0.650
Other	4 (4.2)	3 (4.5)	1 (3.4)	1.000
NBS, median (IQR)	2 (1–3)	2 (1–2)	2 (2–3)	<0.001[a]
Total hospital complications, median (IQR)	1 (1–2)	1 (1–2)	1 (0–3)	0.941
Abdominal injury, n (%)	19 (19.8)	13 (19.4)	6 (20.7)	0.884
Splenic laceration, n (%)	12 (12.5)	9 (13.4)	3 (10.3)	1.000
Sepsis, n (%)	18 (18.8)	14 (20.9)	4 (13.8)	0.413
SSA, median (IQR)	0 (0–1)	0 (0–1)	0 (0–1)	0.541

[a]Comparisons that are statistically significant ($p < 0.05$).
[b]Insufficient data in each category to determine statistically significant comparisons.
[c]Comparisons that demonstrate a trend ($p < 0.10$).
BMI, body mass index; GCS, Glasgow Coma Scale; IQR, interquartile range; ISS, Injury Severity Score; SDH, subdural hemorrhage; SAH, subarachnoid hemorrhage; DAI, diffuse axonal injury; EDH, epidural hemorrhage; IVH, intraventricular hemorrhage; ICH, intracerebral hemorrhage; NBS, neurological burden score; SSA, sepsis, splenic injury, and other abdominal injury score.
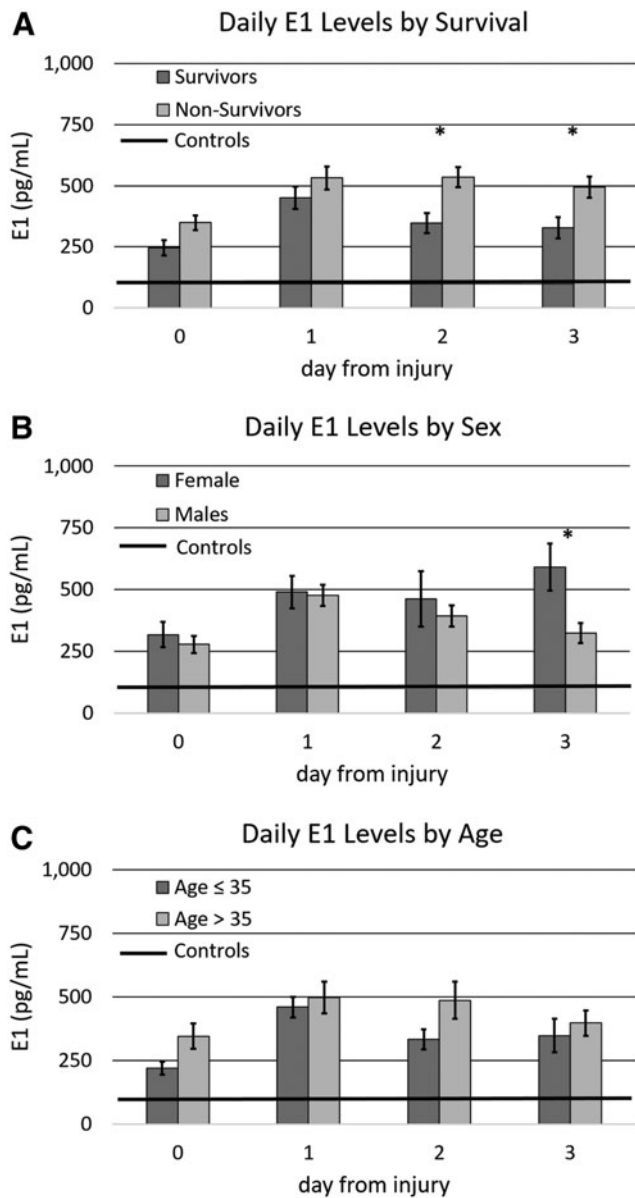
A — Daily E1 Levels by Survival

B — Daily E1 Levels by Sex

C — Daily E1 Levels by Age

FIG. 3. Daily serum estrone (E1) levels after injury by mortality at 1 month, sex, and age with comparison to control levels. **(A)** E1 levels by survival. **(B)** E1 levels by sex. **(C)** E1 levels by age. Means with ±1 SE error bars are shown. *Days with significant differences in E1 by mortality, sex, or age.

significant differences in daily E1 levels by age were noted ($p > 0.05$ all comparisons).

Associations of demographic variables, clinical characteristics, and mortality with E1

The cohort was broken down into tertiles based on cubic spline analysis (E1 low [E1-L], middle [E1-M], and high [E1-H] groups) to represent day 0–3 average levels in the 0th–25th, 25th–75th, and 75th–100th percentiles by using E1 cut points 200.9 pg/mL and 558.0 pg/mL. Clinical and demographic characteristics by E1 tertile are shown in Table 2. There was a trend for GCS to be lower (indicating more severe neurological injuries) among patients in higher E1 tertiles ($p = 0.094$). Individuals in higher E1 tertiles also

had higher rates of abdominal injury (E1-H 37.5%, E1-M 18.8%, E1-L 4.2%; $p = 0.015$). Although presence of sepsis was significantly associated with the E1 tertile ($p = 0.025$), there was a U pattern such that higher rates of sepsis were observed in the E1-H (33.5%) and E1-L (25%) groups compared to the E1-M (8.3%) group. Individuals in higher E1 tertiles also tended to have higher rates of splenic lacerations ($p = 0.084$). Overall, the SSA score increased as E1 tertile increased ($p = 0.012$) (see Fig. 4). Mortality frequency at 1 month was significantly associated with E1 tertile ($p = 0.018$), with rates of 50.0% in E1-H, 29.2% in E1-M, and 12.5% in E1-L. Age, sex, race, BMI, ISS, non-head ISS, and injury types on CT were not associated with 1 month mortality status.

Multivariate regression modeling: E1 association with mortality

E1 tertiles were assessed as a 1 month mortality predictor (Table 3). In addition to age, additional demographic and injury characteristics associated with mortality ($p < 0.2$) were explored in the regression model. An interaction between sex and E1 tertile was explored, but did not significantly contribute to this model. The final model showed that when adjusting for the covariates of age, sex, best in 24 h GCS, and NBS, individuals in the E1-H group, but not the E1-M group, had increased odds for mortality compared with the E1-L group (adjusted OR = 5.656, 95% CI = 1.102–29.045, $p = 0.038$).

T-dependent and T-independent sex hormone steroidogenesis

Mean levels of E2, Andro, and T over the first 72 h post-TBI were assessed by E1 tertile (Table 4). E2 and Andro were positively associated with E1 tertile ($p < 0.001$ and $p = 0.001$, respectively). Average E1, E2, Andro, and T levels are provided in relation to mortality for each hormone and are displayed in Table 5. Non-survivors, compared with survivors, had elevated levels of E1, E2, Andro, and T ($p < 0.05$, all comparisons).

The SEM exploring causal relationships between serum hormones in the aromatization pathway ($n = 92$) is provided in Figure 5. The strongest association in the pathway was the relationship from E1 to E2 ($\beta = 0.83$, SE = 0.18, $p < 0.001$). Other relationships in the pathway that were significant included (Andro to E1 [$\beta = 0.45$, SE = 0.20, $p = 0.02$], Andro to T [$\beta = 0.12$, SE = 0.05, $p = 0.02$] and the reverse: T to Andro [$\beta = 0.15$, SE = 0.05, $p = 0.005$]). T was not a significant contributor to E2, potentially because of its significant reverse pathway relationships to Andro. Moreover, the indirect pathway from Andro to E2, which reflects overall E2 production and considers all relationships in the aromatization pathway, was significant ($\beta = 0.32$, SE = 0.10, $p < 0.001$). Interestingly, post-hoc exploratory SEM analysis (not shown) demonstrated that among survivors, the indirect pathway representing all pathways of E2 production was not significant ($\beta = 0.13$, SE = 0.12, $p = 0.278$). In non-survivors, the indirect pathway from Andro to E2 was significant ($\beta = 0.43$, SE = 0.16, $p = 0.007$), suggesting that larger injury-induced increases in what is primarily E1-mediated E2 production occurs in the setting of mortality.

Steroidogenesis pathway modifier hormones and cytokines: Associations with E1

Average TNF-α, IL-6, progesterone, and cortisol by E1 tertile are provided in Table 4. Progesterone levels increased as E1 tertile increased ($p = 0.003$), suggesting that high progesterone levels may

TABLE 2. CHARACTERISTICS OF TBI COHORT BY E1 TERTILES (LOW, MEDIUM, HIGH)

	E1-L (n = 24)	E1-M (n = 48)	E1-H (n = 24)	p value
Age, mean (SE)	35.1 (3.1)	36.5 (2.4)	41.0 (3.7)	0.505
Sex, men (%)	21 (87.5)	42 (87.5)	17 (70.8)	0.165
Race, n (%)				0.367
Black	1 (4.2)	5 (10.4)	0 (0.0)	
White	23 (95.8)	42 (87.5)	24 (100.0)	
Other	0 (0.0)	1 (2.1)	0 (0.0)	
BMI, mean (SE)	25.7 (1.2)	25.5 (0.7)	26.7 (1.3)	0.906
GCS (best in 24 h), median (IQR)	7 (6–8)	7 (5–7)	6 (3.5–7)	0.094[a]
ISS, mean (SE)	32.1 (1.9)	33.1 (1.5)	37.5 (2.1)	0.218
Non-head ISS, mean (SE)	10.5 (1.5)	10.9 (1.5)	14.6 (2.2)	0.269
Hospital length of stay, mean (SE)	24.0 (2.5)	19.2 (1.7)	16.9 (3.1)	0.068[a][b]
Mechanism of injury, n (%)				
Motor vehicle accident	12 (50.5)	23 (47.9)	13 (54.2)	
Motorcycle	4 (16.7)	10 (20.8)	4 (16.7)	
Falls	3 (12.5)	8 (16.7)	5 (20.8)	
Assault	0 (0.0)	4 (8.3)	1 (4.2)	
Other	3 (12.5)	2 (4.2)	1 (4.2)	
Unknown	2 (8.3)	1 (2.1)	0 (0.0)	
Injury type, n (%)				
SDH	15 (62.5)	33 (68.8)	13 (54.2)	0.476
SAH	16 (66.7)	34 (70.8)	21 (87.5)	0.203
DAI	9 (37.5)	18 (37.5)	6 (25.0)	0.524
EDH	3 (12.5)	10 (20.8)	4 (16.7)	0.675
Contusion	11 (45.8)	20 (41.7)	13 (54.2)	0.604
IVH	9 (37.5)	15 (31.3)	7 (29.2)	0.807
ICH	10 (41.7)	18 (37.5)	5 (20.8)	0.256
NBS	2 (1–2)	2 (1–3)	2 (1.25–2.75)	0.620
Total hospital complications, median (IQR)	1 (1–2)	1 (0–2)	2 (0–3)	0.444
Abdominal injury, n (%)	1 (4.2)	9 (18.8)	9 (37.5)	0.015[c]
Splenic Laceration, n (%)	1 (4.2)	5 (10.4)	6 (25.0)	0.084[a]
Sepsis, n (%)	6 (25.0)	4 (8.3)	8 (33.3)	0.025[c]
SSA, median (IQR)	0 (0–1)	0 (0–1)	2 (1–2.5)	0.012[c]
Mortality, n (%)	3 (12.5)	14 (29.2)	12 (50.0)	0.018[c]

[a]Comparisons that demonstrate a trend ($p < 0.10$).
[b]Insufficient data in each category to determine statistically significant comparisons.
[c]Comparisons that are statistically significant ($p < 0.05$).

TBI, traumatic brain injury; E1, estrone; BMI, body mass index; GCS, Glasgow Coma Scale; IQR, interquartile range; ISS, Injury Severity Score; SDH, subdural hemorrhage; SAH, subarachnoid hemorrhage; DAI, diffuse axonal injury; EDH, epidural hemorrhage; IVH, intraventricular hemorrhage; ICH, intracerebral hemorrhage; NBS, neurological burden score; SSA, sepsis, splenic injury, and other abdominal injury score.
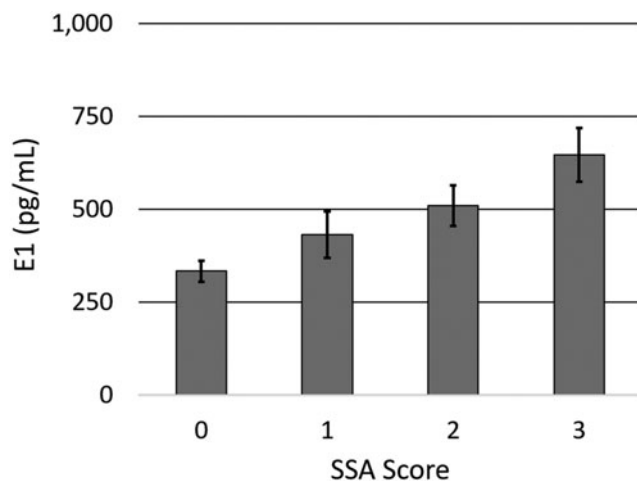

FIG. 4. Estrone (E1) levels by SSA score. E1 significantly increases with SSA score ($p = 0.012$). Means with ±1 SE error bars are shown.

facilitate or enhance steroidogenesis. The aromatase gene transcription factor and serum mortality marker, TNF-α,[62] tended to be positively associated with E1 tertile ($p = 0.056$). Mean IL-6 and cortisol serum levels significantly increased with E1 tertiles ($p = 0.033$ and $p = 0.006$, respectively), suggesting the possibility of elevated aromatase gene transcription among those with higher E1 tertiles.

Discussion

Understanding injury-induced alterations in gonadal and extragonadal steroidogenesis is important in order to understand their role in the systemic response to TBI and in sex hormone associations with TBI outcome. This study is the first to characterize serum E1 levels in a clinical population with severe TBI and to show how these levels relate to other hormones in the steroidogenesis pathway, to relevant demographic and clinical variables, and to mortality status. Notable comparisons with demographic variables include no significant association of E1 with either age or BMI.

TABLE 3. MULTIVARIATE ODDS RATIO ESTIMATES
FOR 1 MONTH MORTALITY

Independent variable	Odds ratio	95% CI		p value
Age	1.044	1.012	1.077	**0.007***
Sex – men	0.586	0.159	2.158	0.421
GCS (best in 24 h)	0.919	0.735	1.150	0.461
NBS	2.720	1.354	5.464	**0.005***
E1-M[a]	2.753	0.623	12.165	0.182
E1-H[a]	5.656	1.102	29.045	**0.038***

[a]E1-L was the reference category.
*Comparisons that are statistically significant ($p < 0.05$).
GCS, Glasgow Coma Scale; NBS, neurological burden score; E1, estrone; M, middle; H, high; L, low.

However, our data do show that TBI results in elevated serum E1 levels compared with control subjects. Additionally, progesterone levels were positively associated with E1 tertiles, suggesting that progesterone levels facilitate E1 production. In a multivariate model controlling for age, sex, best in 24 h GCS, and NBS, the highest tertile of E1 was associated with an increased odds of mortality by 1 month. We also assessed the aromatization pathway and inter-relationships among serum Andro, T, E1, and E2 in the acute period after TBI, including both T-dependent and T-independent pathways for E2 production. It is of note that all four of these hormones were elevated on days 0–3 post-injury in non-survivors compared with survivors, possibly indicating increased sex hormone steroidogenesis in more severe states of systemic stress. These findings are consistent with our prior studies in this cohort, demonstrating worse outcomes in patients with acute elevations in T and E2.[11]

There are limited studies investigating the link among E1, brain injury pathology, and outcomes. One study shows that pharmacological E1 administration to murine cortical neurons subjected to oxidative stress and excitotoxicity significantly reduces neuronal injury.[46,47] Similar to the neuroprotective effects observed with E2 and progesterone administration using *in vivo* models of experimental TBI, E1 therapy also was shown to reduce apoptotic cell death and ischemia following experimental TBI.[45] However, the clinical relationship between E1 and TBI observed in our study parallels the relationship between E1 and insult severity as well as 3 month mortality clinically after aneurysmal SAH.[27] Also, our findings with E1 and mortality are consistent with our previous report showing that higher E2 levels are associated with 6 month mortality and poor global outcome.[11] Together with the current report, the data suggest that endogenous systemic estrogen asso-

ciations represent unique systemic responses to injury that are independent of the neuroprotective sex hormone (progesterone/E2) effects observed with pharmacological dosing in animal models of isolated CNS injury, in which extracerebral trauma, systemic response to injury, and complications including sepsis and critical illness are not a part of model preparation.[23,48] It is of note that we have previously found that individuals with higher E2 to T ratios in cerebrospinal fluid (CSF) following TBI have better global outcome, suggesting that traditional neuroprotective benefits of E2 production and/or T consumption are observed in the CNS.[53] Similarly, future CSF analysis of E1 relationships to outcome after TBI is warranted.

Mechanistic links underlying E1 associations with poor outcomes following TBI have not yet been described, but may have to do with the non-neurological consequences of TBI such as hypotension and sepsis,[4,50,63,64] which are commonly associated with mortality among critically ill individuals. Studies have identified significant associations between elevated E1 with septic shock and vasodilation.[23,48] Given our previous reports of HPA activation after TBI,[11] the innate systemic inflammatory response that occurs after TBI,[49] and the prevalence of sepsis and other major infections,[4] it may be that the elevated inflammatory mediators that accompany these responses such as TNF-α,[65] cortisol,[66] and IL-6[67] also amplify aromatase activity in peripheral adipose tissues, where the literature suggests that there is a preferential increase in Andro aromatization to E1.[30,33,34]

Increased TNF-α in adipose tissue is associated with upregulation of promoter I.4 responsible for the expression of the aromatase gene.[51,68,69] Upstream from this promoter, exists a glucocorticoid response element necessary for gene expression.[35] Further upstream is a γ interferon-activating sequence that responds to activation of the Jak tyrosine kinase and signal transducer and activator of transcription (STAT) factor pathway.[36] Studies show that IL-6 can activate the Jak/STAT pathway to increase aromatase expression in adipose tissue, provided that soluble IL-6 receptor is available.[36] The cytokine and hormonal milieu following both septic and non-septic inflammation following TBI may therefore contribute to increased aromatase activity and consequently elevated E1 production. Indeed, our data show that mean levels of TNF-α, cortisol, and IL-6 are associated with higher E1 tertiles. Therefore, E1 may be a common marker of poor outcomes in both TBI and sepsis, in part because of its association with more severe inflammation.

It is not surprising that E1 levels in our cohort do not reflect the established norms (that postmenopausal aging women show an increase in E1[70,71] and aging men reflect the opposite trend[72,73]) given the hormonal dysregulation observed as a consequence of

TABLE 4. HORMONES AND CYTOKINES BY E1 TERTILES

	n	E1-L	E1-M	E1-H	p-value
TNF-α, pg/mL (SE)	80	9.4 (0.9)	16.3 (5.2)	13.8 (1.6)	0.051[a]
IL-6, pg/mL (SE)	80	252.4 (52.6)	249.4 (62.2)	618.5 (210.5)	0.033[b]
Progesterone, ng/mL (SE)	95	1.9 (0.4)	2.4 (0.4)	4.6 (1.1)	0.003[b]
Cortisol, ng/mL (SE)	95	191.2 (12.0)	209.5 (10.8)	296.5 (26.2)	0.006[b]
E2, pg/mL (SE)	92	36.1 (3.6)	73.6 (6.3)	227.0 (45.0)	<0.001[b]
Andro, ng/mL (SE)	92	1.3 (0.2)	2.2 (0.2)	2.6 (0.4)	0.001[b]
T, ng/mL (SE)	92	1.0 (0.2)	1.3 (0.2)	1.3 (0.2)	0.329

[a]Comparisons that demonstrate a trend ($p < 0.10$).
[b]Comparisons that are statistically significant ($p < 0.05$).
E1, estrone; TNF, tumor necrosis factor; IL, interleukin; E2, estradiol; Andro, androstenedione; T, testosterone.

Table 5. Serum E1, E2, Andro, and T Average Day 0–3 Levels by 1 Month Mortality

	Total (n = 92)	Survivors (n = 64)	Non-survivors (n = 28)	p value
E1, pg/mL (SE)	384.3 (25.7)	337.7 (27.6)	490.8 (51.1)	0.008[a]
E2, pg/mL (SE)	104.6 (14.4)	85.8 (16.8)	147.7 (26.3)	0.001[a]
Andro, ng/mL (SE)	2.1 (0.2)	1.7 (0.1)	2.9 (0.4)	0.003[a]
T, ng/mL (SE)	1.3 (0.1)	1.1 (0.1)	1.6 (0.3)	0.039[a]

[a]Significant differences by mortality status at 1 month ($p < 0.05$).
E1, estrone; E2, estradiol; Andro, androstenedione; T, testosterone.

TBI.[11] The lack of positive association between E1 and age in our cohort is interesting in the context of our prior work demonstrating elevated acute levels of other sex hormones (progesterone and E2) in older patients following TBI,[11] but may be the result (in part) of differences in data presentation in each report (e.g., mean value vs. trajectory group membership). However, our previous work in this same published report shows no age differences in T, which like E1, is an immediate precursor for E2.[11] When taken together, evidence from the previous and this current report show that neither immediate precursor for E2 (i.e., E1 and T) were affected by age. Regardless of age, previous work suggests that aromatization in extragonadal tissues is a significant source of estrogen production. As such, typical age effects on neuroendocrine production are less likely to influence the acute hormone profiles that we have reported in this cohort.

Multiple studies have linked elevated E1 levels to obesity because of the increased aromatization occurring in adipose tissue.[70,71,74] Given that acute elevations in progesterone are a consequence of TBI[11] and that progesterone can be utilized by adipose tissue for Andro and, subsequently, E1 production,[75] it is somewhat surprising increased BMI is not associated with increased E1 production following injury. However, BMI is neither the most accurate marker for obesity nor does it accurately predict physiological consequences of increased adiposity,[76–78] factors which may explain this lack of association. Future studies may benefit from examining more sensitive measures of adiposity, such as waist circumference.[78]

Interestingly, our data show that although not associated with 1 month mortality, sepsis was associated with higher E1 levels after TBI. This finding may support the idea that sepsis burden may be a unique contributor to systemic injury-induced steroidogenesis apart from the innate inflammatory response associated with severe TBI in general. We observed a similar positive association between rates of abdominal injury and E1. Given that abdominal trauma can induce inflammatory responses that includes IL-6 cytokine production,[79,80] its association with E1 may be a consequence of increased inflammatory cytokine induced aromatization. Further, those with splenic laceration tended to have higher E1 production, which is interesting, given that early splenectomy has been shown in one report to reduce TBI-associated mortality and improve cognitive performance in an animal model of severe TBI.[81] Taken together, the SSA score, representing the sum of the presence of sepsis, splenic laceration, and other abdominal injury, demonstrated significant positive association with E1 levels and E1 tertiles in our population, further reinforcing that extracerebral inflammatory burden may contribute to elevated E1 levels.

Serum E1 and E2 associations with mortality may be as much a consequence of a more severe clinical disease state as it is a direct result of E1 and E2 effects on systemic physiology. Reports suggest that 40% of single-cause acute mortality associated with TBI results from the systemic response to TBI and/or concurrent extracerebral trauma known as NNOD, and that 85% of mortality is in part caused by non-neurological conditions/complications.[5] The case for estrogen-driven systemic injury and NNOD in response to TBI and its associated injury complex is plausible. Experimental studies demonstrate direct actions of E2 in sepsis and vasodilation, which are potential non-neurologic complications of TBI. In addition to E1 vasodilatory effects via Ca^{2+} channel receptors and NOS activation,[82,83] E2 induces vasodilation through both nitric oxide- dependent and hydrogen sulfide-dependent pathways.[84–87] A significant portion of TBI mortality is related to vascular system compromise that leads to NNOD complications, including arrhythmias, myocardial ischemia, neurogenic pulmonary edema, congestive heart failure, and acute kidney injury,[4–6] much of which can arise from sympathetic nervous system (SNS) overactivity and dysfunction.[9,88,89] SNS-innervated peripheral lymphoid tissue can perpetuate the inflammatory response, including ongoing IL-6 and TNFα
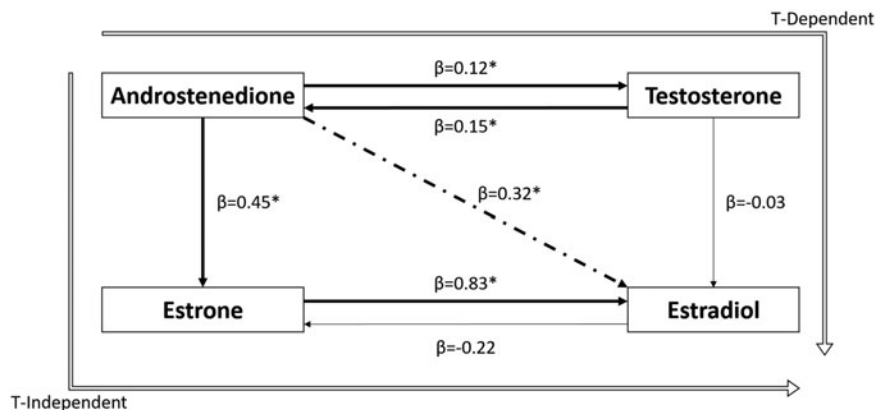

FIG. 5. Sex hormone structural equations model (SEM) in all subjects (n = 92), Androstenedione (Andro)/Estrone (E1), E1/Estradiol (E2), and forward and reverse pathways for testosterone (T)/Andro were significant. The indirect Andro to E2 path was significant as well. Weighted lines represent significant associations in the pathway ($p < 0.05$). The dotted line represents the indirect Andro to E2 path. Unfilled lines represent the T-independent and T-dependent pathways of E2 production from Andro. *Significant paths ($p < 0.05$).

production,[8,9] which fuels E1 production from Andro. As the metabolic product of E1, E2 production can have direct effects on SNS-mediated inflammation by blocking adrenergic reuptake,[9,90] potentiating the sympathetic response to injury, and indirect effects through TNFα that have potent vasodilator effects on systemic vasculature.[91,92] Together, estrogen effects on inflammation and vasodilation are viable mechanisms by which elevated systemic estrogen production may contribute to NNOS and worsen TBI outcomes.

Structural equations modeling, a causal inference framework, was used to derive estimates regarding the magnitude and direction of the causal relationships among Andro, T, E1, and E2. The proposed model, with the T-dependent and T-independent paths, is highly grounded in known hormone biology. The structural model displays the interrelationships among a set of observed hormone values as a succession of structural models, which is parallel to running a series of regression equations.[93] The effects estimated from the equation represent causal relationships in instances for which the key assumptions of causality are met, which include temporality, normality, linearity, no unmeasured confounding, or common effects. In all patients, independent of survivorship, $A \rightarrow E1$, $E1 \rightarrow E2$, $T \rightarrow Andro$, $Andro \rightarrow T$, and the indirect $A \rightarrow E2$ were significant pathways. Both forward and reverse pathways in the Andro/T interconversion had similar effect size, indicating relative equilibrium between the oxidative and reductive pathways between these hormones. Further, overall, T-independent aromatization to E2 via $Andro \rightarrow E1$ and $E1 \rightarrow E2$ was significant, as was $Andro \rightarrow E2$, which is a measure of "all-cause aromatization." That all-cause aromatization to E2 was associated with mortality is consistent with prior studies in major illness indicating increased estrogen production through androgen consumption is associated with worse outcomes.[20,23,24,31] The significant conversion of E1 to E2 in non-survivors compared with survivors may suggest a change in the enzyme activity responsible for their interconversion, 17β-HSD. Little is known regarding 17β-HSD function in relation to trauma, inflammation, or sepsis. Isoforms of 17β-HSD have preferential activity toward the oxidative or reductive pathway.[94] Thus, these enzymes do not drive steroid flux in one direction, but instead, support functional equilibria among intact cells, reflecting thermodynamically driven steroid distributions.[28,94] One potential interpretation of the relationships described is that injury is associated with an equilibrative shift away from T-dependent aromatization, and mortality is associated with a stronger equilibrative shift of this enzyme toward oxidative pathways that facilitate T-independent estrogen (E1 and E2) production.

These data on systemic aromatization associations with mortality may help explain clinical trial failures examining progesterone therapy following TBI.[95,96] It is of note that previous explanations for these failures have been proposed and include: inappropriate classification of TBI with oversimplified unidimensional scoring systems, non-optimized treatment dosing and duration, and heterogeneity of patient population and injury characteristics.[97] However, we propose that physiological mechanisms may exist for progesterone's clinical failure. Indeed, prior characterization of endogenous sex hormone steroidogenesis following TBI demonstrates that progesterone has a significant negative impact on mortality through its effect on E2.[11] We demonstrate that systemic production of E2 from Andro in all patients is elevated following TBI, and that this occurs predominantly through the T-independent pathway. Exogenous progesterone administration could amplify T-independent E2 production and increase systemic E2 and T levels, both of which are associated with poor outcomes following brain injury.[11,39] Although rodent models of TBI do not recapitulate the extracerebral trauma complex, the

critical illness, and the period of coma observed clinically after severe TBI, recent studies in a rodent weight drop model do show elevated plasma progesterone levels for males and an transient increase in E2 among female rats acutely after injury,[98,99] findings that are consistent with clinical literature and suggestive that peripheral steroidogenesis in acute response to TBI may be a mechanistic consideration for future study in animal models of TBI that include other concomitant injury as a part of the model. We hypothesize that given progesterone's success in isolated experimental TBI studies, patients with TBI who have a less-pronounced systemic amplification of the aromatization pathway may be more likely to benefit from progesterone as a neuro-therapeutic. Further examination of sex hormone steroidogenesis in patients from prior clinical trials utilizing progesterone in TBI would be useful in exploring this hypothesis.

Study limitations include the lack of generalizability of results. Given known variation in predilection for E1 production in aging men and postmenopausal women,[71–73] E1 patterns following TBI may represent different magnitudes of association with outcome based on demographic characteristics. Although this was not demonstrated in our study, subpopulations based on these characteristics were too small to draw definitive conclusions from. However, recent trends suggest increased age, and associated comorbidity with TBI, which may influence NNOD, complications, and systemic biomarkers reflecting these patient characteristics.[100,101]

Another limitation is that sepsis was defined using ICD-9 codes, in contrast to using a state of the art prospective case-based definition.[102] Also, prospective temporal assessment of sex steroids and infection type/treatment, systemic inflammatory response syndrome (SIRS), shock, and pressor requirement would be a useful future direction. Finally, the study is limited in that we did not delineate the cause of death in this cohort and whether or not mortality was primarily the result of neurological causes or related to extracerebral injury complex or response to the TBI.

Conclusion

E1 is as an intriguing mortality marker after TBI because it may directly impact the non-neurologic consequences of TBI and be an indicator of a T-independent pathway to E2 production. Although laboratory studies indicated that E1 may improve TBI outcome,[45,46] this study demonstrated that E1 is associated with worse outcomes clinically, further reinforcing the paradox observed in pre-clinical and clinical data in TBI. Future work conducting a clinical CSF analysis of E1 may inform differences between pre-clinical and clinical data. Further, future research into the therapeutic potential of aromatase inhibitors in TBI should be explored, given the improved immune response in experimental models of trauma and hemorrhage observed with these drugs[103,104] and the negative association between TBI outcomes and estrogen production outlined here. Injury severity associations with E1 could also then be explored in this model.

Acknowledgments

Author Disclosure Statement

No competing financial interests exist.

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